



Biochar, but not soil microbial additives, increase the resilience of urban plant species to low water availability

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Abstract

Impervious surfaces that characterise urban areas can make them harsh, water-limited places for plants to grow. To help alleviate plant stress under these challenging drought conditions, a range of soil additives can be utilised. Although well-studied individually, our understanding of the interaction between different soil additives in alleviating drought stress in urban areas is rudimentary. The aim of this study was to (1) assess the growth of urban horticultural plant species under drought stress and (2) determine whether drought stress in these species is alleviated by the use of soil additives, both individually and in combination. We grew six plant species (three trees, three graminoids) commonly planted in Australian urban areas under two watering regimes (drought-stressed, well-watered) and four soil additive treatments (no additive, microbial additive only, biochar only, microbial additive and biochar), and assessed their performance. We found that drought stress significantly reduced the growth of the six study species. Surprisingly, this decrease in growth was not reduced with the use of soil additives, despite biochar increasing soil water content and mycorrhizal colonisation when used in combination with the microbial additive. However, the addition of biochar significantly delayed the visual onset of drought stress across all species. Our results show that soil additives can be used as a cost-effective management strategy to increase plant resilience to drought stress in urban areas.

Keywords Climate change · Inoculation · Microbiome · Soil amendments · Urban greening

Introduction

It is increasingly recognised that urban forests (i.e. the sum of all plants and pervious soils in an urban area; Escobedo et al. 2011) provide a range of social, health, economic and environmental benefits that can substantially improve livability and thus human well-being (Roy et al. 2012; Botzat et al. 2016; Salmond et al. 2016; Livesley et al. 2016). However, the challenging environmental conditions that often prevail in urban areas make it difficult for plants to survive and thrive. For example, urban areas are often water-limited because the impervious surfaces that characterise

them reduce rainfall infiltration (Mejía and Moglen 2010; Yao et al. 2016; Shao et al. 2019) as well as exacerbate the microclimatic conditions that facilitate soil water loss (e.g. reduced humidity, increased temperature, increased wind activity) (Sieghardt et al. 2005; McClung et al. 2018; Wang et al. 2019). These water limitations may be further exacerbated by the projected increase in the frequency and intensity of extreme drought events under climate change in some regions (Seneviratne et al. 2012). Despite plants having a range of coping strategies (e.g. stomatal closure, investment in root growth, leaf senescence) for low water availability (Chaves et al. 2003), urban forests are more vulnerable to the impacts of drought stress than natural forests (Gillner et al. 2014; Savi et al. 2015; Nitschke et al. 2017; Bialecki et al. 2018; Toscano et al. 2019; Miller et al. 2020).

Given the vulnerability of urban forests to drought stress, a high priority should be placed on enhancing their resilience to such stress, so they can continue providing key ecosystem services into the future (Güneralp et al. 2015; Raymond et al. 2017). The most effective strategy to improve

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the resilience of urban forests to drought stress is to transition species selection toward a more drought-resilient suite of species (Sjöman et al. 2015; Vahmani and Ban-Weiss 2016; Espeland and Kettenring 2018). However, this does not always guarantee success, so other strategies (e.g. soil amendments, microbial additives) aimed at improving the hydraulic properties of the soil, which is the critical interface of plant-water relations, can be employed (Kirkham 2014).

The soil microbial community plays a key role in the functioning of urban forests (Rumble and Gange 2017; John et al. 2017; Molineux et al. 2017; Fulthorpe et al. 2018; Hoch et al. 2019; Xie et al. 2020; see van Geel et al. 2019 for exception). One critical role performed by the soil microbial community is improving plant-water relations, which in turn can enhance plant resilience to drought stress (Kilvin et al. 2013; Mayerhofer et al. 2013; Jayne et al. 2014). For example, arbuscular mycorrhizal fungi have been shown to reduce drought stress by allowing the plant to more thoroughly explore the soil for water, improving stomatal conductance and increasing anti-oxidant enzyme activity (Jayne et al. 2014; Augé et al. 2015; Bahadur et al. 2019; Goyal et al. 2020). However, the soil microbiome in urban areas often has less diversity and richness than in non-urban areas (Pavao-Zuckerman 2008; Epp Schmidt et al. 2017; Ferreira et al. 2018). Therefore, soil microbial additives may prove a useful tool in supplementing the urban soil microbiome, thus enhancing the resilience of urban forests to drought stress (Fini et al. 2011; see Schröder et al. 2019 for exception).

Another useful soil additive that can be used to improve plant-water relations in urban forests is biochar. Biochar is a carbon-rich material that is formed by the pyrolysis of plant biomass under a limited oxygen supply (Weber and Quicker 2018; Panahi et al. 2020). The effectiveness of biochar in reducing drought stress in urban plant species has been well documented (Cao et al. 2014; Huang et al. 2020; Somerville et al. 2020; Yoo et al. 2020; see Dai et al. 2020 for biochar effects on plant growth). In addition, biochar also creates a more favourable environment for soil microbes to thrive (e.g. greater water retention, increasing carbon and nutrient supply, detoxification of allelochemicals and more refugia), which may further alleviate drought stress (Warrack et al. 2007; Palansooriya et al. 2019). However, the

interaction between biochar and soil microbial additive use (Scharenbroch et al. 2013; Chen et al. 2018), and the subsequent effect it may have on urban plant resilience to drought stress, has yet to be investigated.

The aim of this study was two-fold: (1) to assess the growth of urban horticultural plant species under drought stress and (2) to determine whether drought stress in these species is alleviated by the use of soil additives (microbial additive, biochar), both individually and in combination. To address these aims, we grew six plant species (three trees, three graminoids) that are commonly planted in Australian urban areas in a fully factorial glasshouse experiment with two treatments: watering regime (drought-stressed, well-watered) and soil additive (no additive, microbial additive only, biochar only, microbial additive and biochar). After 12 weeks exposed to these treatments, the growth performance and visible stress of each plant were measured. We hypothesised that:

- 1) Individually, the addition of the soil microbial additive and biochar will increase mycorrhizal colonisation and soil water retention, respectively, thus alleviating the effects of drought stress on plant performance.
- 2) The addition of the soil microbial additive in conjunction with biochar will alleviate the effects of drought stress on plant performance beyond the individual effects of each additive.

Methods

Species selection and plant preparation

We selected three evergreen tree species and three graminoid species that are commonly planted in Australian urban areas (Table 1). All the species are considered to be fast-growing and moderately drought-resilient, according to nursery industry classifications (Tabassum et al. 2021). For each species, 63 tubestock plants (pot size: 0.25 L, h = 120 mm, w = 50 mm) of similar size and age (within species) were sourced from commercial suppliers (Downes Wholesale Nursery, Theresa Park, NSW, Australia; Botanica Nurseries, Silverdale, NSW, Australia).

Table 1 Taxonomy, growth form and native range of each study species

| Species | Common name | Family | Growth form | Native range |
|---------------------------------------------------|-----------------------|----------------|-------------|--------------|
| <i>Elaeocarpus reticulatus</i> Sm. | Blueberry ash | Elaeocarpaceae | Tree | Australia |
| <i>Hymenosporum flavum</i> (Hook.) F.Muell. | Native frangipani | Pittosporaceae | Tree | Australia |
| <i>Waterhousea floribunda</i> (F.Muell.) B.Hyland | Weeping lilly pilly | Myrtaceae | Tree | Australia |
| <i>Liriope muscari</i> (Decne.) L.H.Bailey | Big blue lilyturf | Asparagaceae | Graminoid | East Asia |
| <i>Lomandra longifolia</i> Labill. | Spiny-headed mat-rush | Lomandraceae | Graminoid | Australia |
| <i>Ophiopogon japonicus</i> (Thunb.) Ker Gawl. | Mondo grass | Asparagaceae | Graminoid | East Asia |

Within each species, the plants were arranged in order of size to ensure each treatment had a similar size distribution. For the tree species, size was measured based on plant height and stem diameter at 10 cm while for the graminoid species, leaf number was used. Seven plants per species were allocated based on size to the pre-treatment harvest (i.e. pre-treatment plants) and each of the eight treatment combinations (i.e. experimental plants; treatments described below) to ensure an even distribution of plant sizes across treatments. Once allocated, the plants were bare-rooted and transplanted into cylindrical pots (2.7 L; $h=150$ mm, $d=150$ mm) containing 2.5 L of organic potting mix (Australian Growing Solutions, Tyabb, VIC, Australia) with 10 g of slow-release fertiliser (16 N:4.4P:8.3 K; Osmocote, Scotts Australia Pty Ltd, Bella Vista, NSW, Australia) homogenised into it. Organic potting mix was used for the experiment instead of urban soil for two reasons. Firstly, urban plantings are often backfilled with commercially supplied soil (e.g. organic potting mix) rather than urban soil (City of Sydney 2011). Secondly, the abiotic soil properties of organic potting mix would likely be less variable than urban soil, hence, reducing the number of confounding factors that may influence the treatment effects. Note that each pot allocated to the biochar treatments had 250 g of granulated biochar (10% of the organic potting mix volume; see Table S2 for the full list of biochar properties) homogenised into the potting mix. Plants were then allowed to establish for four weeks, getting mist watered thrice daily for three minutes.

Pre-treatment harvest and experimental design

After the four-week establishment period, baseline growth trait measurements of each plant were recorded. For the tree species, plant height and stem diameter at 10 cm were measured. For the graminoid species, leaf and tiller number were counted. Once the growth traits were recorded, the pre-treatment plants were harvested, oven-dried at 60 °C for 48 h and weighed using an analytical balance (Mettler Toledo, Port Melbourne, VIC, Australia). The purpose of the pre-treatment harvest was to determine the relationship between the growth traits and dried biomass for each species, thus allowing the initial (i.e. pre-treatment) biomass of the experimental plants to be non-destructively calculated.

Following the pre-treatment harvest, the treatments were applied to the experimental plants. The plants were grown in a fully factorial glasshouse experiment under two treatments: watering regime (drought-stressed, well-watered) and soil additive (no additive, microbial additive only, biochar only, microbial additive and biochar). This gave a total of eight treatments, resulting in 336 pots for the experiment (6 species \times 8 treatments \times 7 replicates).

For the watering regime treatment, the well-watered plants were connected to an automatic drip watering system that delivered 30 mL of water per pot thrice daily through spikes. The drought-stressed plants, on the other hand, were placed on an apparatus developed by Marchin et al. (2020) that lowers volumetric soil water content (VSWC) to the desired level and then maintains it over time. Briefly, this apparatus functions by the plants using capillary action to draw water up through floral foam that has low water permeability. Using this apparatus, water levels were drawn down 15 mm per day from a height of 210 mm (i.e. top of the floral foam) until no water remained in the tubs. This resulted in a 14-day draw-down period. After the draw-down period, the plants were left to grow for a further 10 weeks under constant drought stress ($\sim 1\text{--}5\%$ VSWC). During the experiment, the VSWC of each pot was measured on a bi-weekly basis using a Hydrosense II Portable Soil Moisture System (Campbell Scientific Australia Pty Ltd, Garbutt, QLD, Australia).

For the soil microbial additive treatment, a broad spectrum commercially available microbial mixture (see Table S1 for the full list of microbes and their concentrations) was diluted in water (0.70 g/L), with 120 mL of this solution being applied on a fortnightly basis to each plant designated to receive the treatment, as per the manufacturer's instructions. The plants that did not receive the soil microbial additive treatment received 120 mL of water. The application of the biochar treatment is described above.

Each treatment was evenly split at the species-level between two climate-controlled glasshouses at the Plant Growth Facility at Macquarie University (North Ryde, NSW, Australia). The temperature of the glasshouses was set to a minimum (night-time) of 19 °C and a maximum (day-time) of 24 °C, which was continuously maintained by a fan coil unit using a water cooling and heating system. Temperature, relative humidity (glasshouse 1 = $62 \pm 2\%$, glasshouse 2 = $65 \pm 2\%$ at 1400 h) and photosynthetically active radiation (glasshouse 1 = 342 ± 28 mol $\text{m}^{-2}\text{s}^{-1}$, glasshouse 2 = 447 ± 61 mol $\text{m}^{-2}\text{s}^{-1}$ at 1400 h) were monitored continuously using a MultiGrow controller system (Autogrow Systems, Auckland, New Zealand). Plants were sprayed fortnightly with eco-oil insecticide to control insect outbreaks (Organic Crop Protectants, Clayton, VIC, Australia).

During the experiment, the drought-stressed plants were visually assessed for signs of stress twice weekly. For *H. flavum* and *W. floribunda*, leaf wilting was used to determine if a plant was drought-stressed or not. The difference in appearance between healthy and wilting leaves in these two species was distinct, so misclassification was not possible. For the remaining four species, leaf necrosis ($> 25\%$ leaf area) was used. Typically, the visual onset of drought stress occurred when the VSWC dropped below 2%.

Harvesting and mycorrhizae staining

At the end of the experiment, the growth traits of the plants that were measured pre-treatment were once again measured. The plants were then harvested using the same method as the pre-treatment harvest. Relative growth rate (RGR, g/day) was calculated for each plant using the formula:

$$RGR = \frac{Final\ biomass - Final\ biomass - pre}{84\ days} \quad (1)$$

Before oven-drying the biomass of the plants, a clipping of fine root biomass was taken from each plant and preserved in 80% ethanol solution to measure mycorrhizal colonisation. To do this, root clippings were stained following a modified method of Ho-Plágaro et al. (2020). Briefly, for each plant, three 1–2 cm long segments of root were randomly selected from the clipping. These segments were submerged in 1 mL of 10% KOH and held at 100 °C in a water bath for 45 min to remove the cell cytoplasm and nuclei. Following this, the segments were washed twice in reverse osmosis water before being submerged in 1 mL of 0.1% HCl for 1 h to neutralise the KOH, so the staining agent could react with the tissues. The segments were removed from the HCl and without rinsing, submerged in 1 mL of 0.05% Trypan Blue in 90% lactic acid while being held at 100°C in a water bath for 30 min. Finally, the segments were rinsed twice with reverse osmosis water and then submerged in 90% lactic acid for 12 h before being mounted on a microscope slide and covered with a coverslip. Using a light microscope (BX53, Olympus, Tokyo, Japan) with a digital camera, three images were taken along each segment using ×40 magnification. The total area of mycorrhizal colonisation in each image was measured using the colour threshold function in ImageJ (Schneider et al. 2012).

Data analysis

To determine if VSWC differed between the watering regime × soil additive treatments, a three-way repeated-measures ANOVA was used. The fixed factors were watering regime (drought-stressed, well-watered), soil additive (no additive, microbial additive only, biochar only, microbial additive and biochar) and growth form (tree, graminoid). Species was designated as a random factor and nested within growth form. The repeated measure in the model was the individual plants.

To determine if the RGR and mycorrhizal colonisation differed between the watering regime × soil additive treatments, a nested general linear model was used. The fixed factors were watering regime, soil additive and growth form. Species was designated as a random factor and nested

within growth form. The same analysis was performed for each growth trait with the exception of growth form being removed from the model. Species was the third fixed factor in these models.

To determine if the soil additive treatments delayed the visual onset of stress in the drought-stressed plants, a nested general linear model was used. The fixed factors were soil additive and growth form. Species was designated as a random factor and nested within growth form.

All statistical analyses were conducted using R version 3.5.2 (R Core Team 2017), with the significance level set at 0.05.

Results

There was a significant interaction between the watering regime and soil additive treatments for VSWC ($F_{3,7354}=46.79$, $p<0.001$). Post-hoc analyses revealed that the well-watered pots had significantly higher VSWC than the drought-stressed pots (Fig. 1a). Further, the pots containing biochar (biochar only, microbial additive and biochar) had significantly higher VSWC than the no additive and microbial additive only pots (Fig. 1b). Within the drought stress treatment, the microbial only pots had significantly higher VSWC than the pots containing no additive.

There was also a significant interaction between growth form and the soil additive treatments for VSWC ($F_{3,7354}=3.17$, $p=0.023$). Post-hoc analyses revealed that within the biochar treatments, the trees grown under the microbial additive and biochar treatment had significantly lower VSWC than the other growth form × biochar treatment combinations.

There was no interaction between watering regime, soil additive and growth form for RGR across all species (Table S4). However, there was a significant interaction between watering regime and growth form ($F_{1,314}=49.61$, $p<0.001$; Fig. 2), with the well-watered trees having higher RGR than their drought-stressed counterparts. In contrast, the RGR of the graminoids did not significantly differ between the watering regime treatments. RGR did not significantly differ between the soil additive treatments ($F_{3,314}=2.29$, $p=0.078$; Fig. S1).

There was no interaction between the watering regime and soil additive treatments on the growth traits of the trees or graminoids (Tables S5, S6, S7 and S7). However, the well-watered trees were significantly taller ($F_{2,143}=27.55$, $p<0.001$; Fig. 3a) and had greater stem diameter ($F_{2,143}=13.89$, $p<0.001$; Fig. 3b) than their drought-stressed counterparts. Similarly, the well-watered graminoids produced more leaves ($F_{1,143}=98.34$, $p<0.001$; Fig. 3c) and tillers ($F_{1,143}=28.84$, $p<0.001$; Fig. 3d) than

Fig. 1 The soil water content for each (a) watering regime and (b) soil additive treatment across the study species from the beginning of the drought stress treatment period until the end of the experiment. Note that the first 14 days were the draw-down period for the drought-stressed plants. Soil additive abbreviations: B = biochar only, M = microbial additive only, M + B = microbial additive and biochar, N = no additive

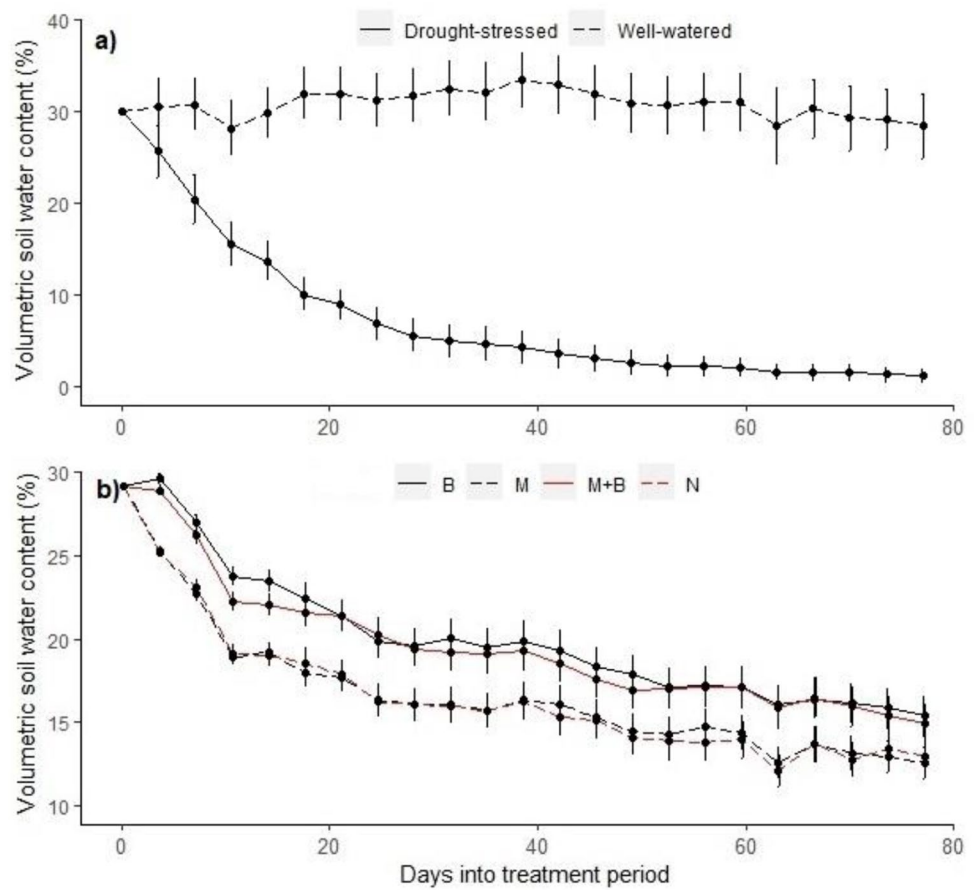
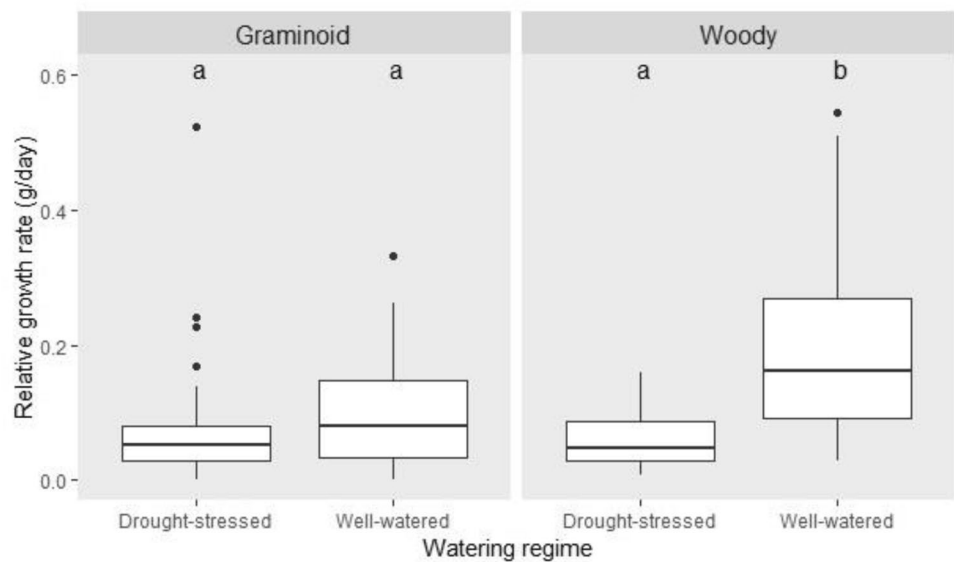


Fig. 2 The relative growth rate for each watering regime across the study species. Boxplots display the middle 50% of the data (interquartile range, IQR). Within the box, the horizontal bar represents the mean value. Vertical bars represent one standard error, while outliers are indicated by dots. Letters indicate significant differences at $p < 0.05$



their drought-stressed counterparts. The trees grown in the microbial additive and biochar treatment were significantly shorter than trees grown in the other soil additive treatments ($F_{3,143}=3.66$, $p=0.014$; Fig. 4a). The trees grown

in the biochar only treatment had a smaller stem diameter ($F_{3,143}=4.20$, $p=0.007$; Fig. 4b) than trees grown in the other soil additive treatments. The soil additive treatments

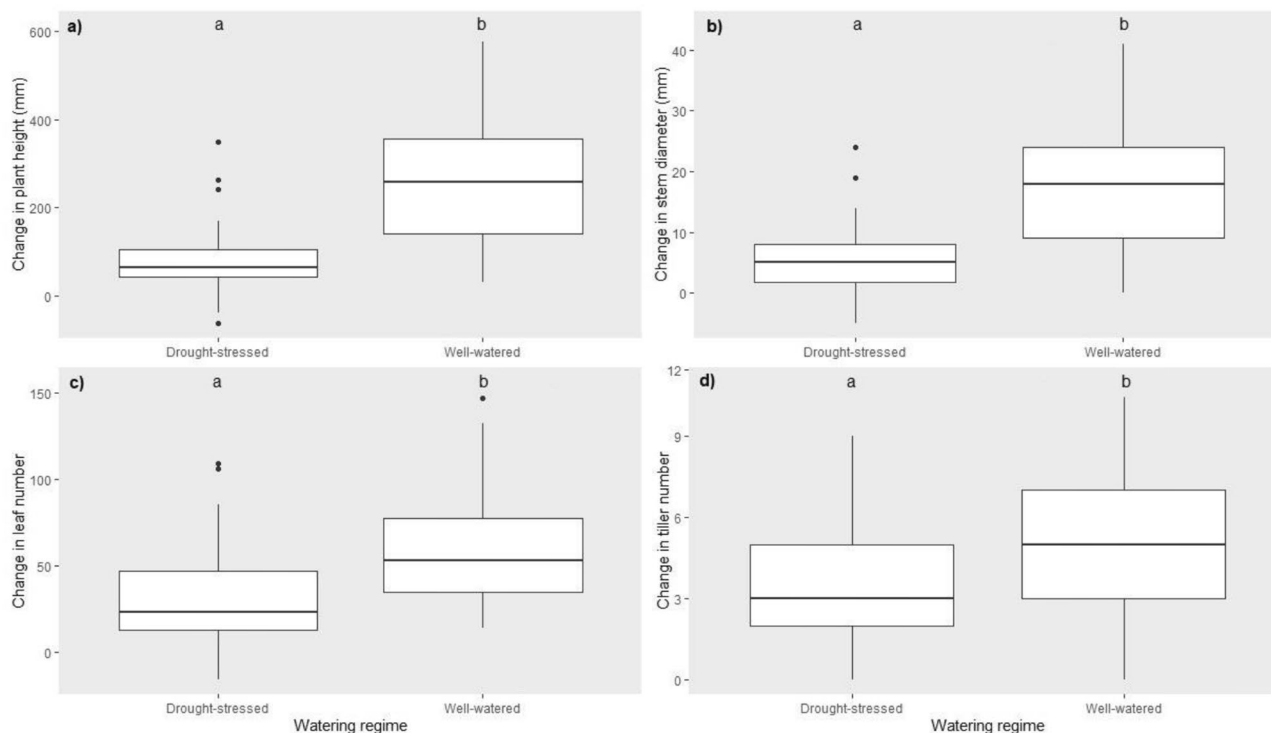


Fig. 3 The growth traits of the trees and graminoids for each watering regime treatment. Boxplots display the middle 50% of the data (interquartile range, IQR). Within the box, the horizontal bar represents the

mean value. Vertical bars represent one standard error, while outliers are indicated by dots. Letters indicate significant differences at $p < 0.05$

did not significantly affect the growth traits of the graminoids (Tables S5 and S6).

There was no interaction between watering regime, soil additive and growth form on mycorrhizal colonisation across all species (Table S9). However, there was a significant interaction between watering regime and growth form ($F_{1,313}=7.69$, $p=0.006$; Fig. 5a), with the well-watered trees having greater mycorrhizal colonisation than the other watering regime \times growth form combinations. Further, plants grown under the soil microbial additive and biochar treatment had significantly greater mycorrhizal colonisation than plants grown in the other soil additive treatments irrespective of the watering regime ($F_{1,313}=7.89$, $p < 0.001$; Fig. 5b).

For the drought-stressed plants, the use of biochar significantly delayed the visual onset of stress compared to the no additive and microbial additive only treatments across all species ($F_{3,155}=20.55$, $p < 0.001$; Fig. 6).

Discussion

We found that drought stress reduced the growth of our study species. This result, coupled with the fact that urban areas are often water-limited, highlights the need to increase

the resilience of urban forests to drought stress to ensure that they continue to deliver key ecosystem services. Currently, several management strategies (e.g. irrigation, species selection) are gaining traction in urban forestry to achieve this goal (Symes and Connellan 2013; Sjöman et al. 2015), with soil additives being amongst the most promising and cost-effective.

Numerous studies have reported the capacity of biochar to increase soil water retention (see meta-analysis Razzaqhi et al. 2020). We found this to be the case in our study, with the drought-stressed and well-watered pots containing biochar having on average 31% and 17% greater VSWC, respectively, than the pots not containing biochar. Although this increase in VSWC did not alleviate the impacts of drought stress on the growth of the study species, it did delay the visual onset of drought stress across all species. That is, the drought-stressed plants grown in biochar displayed visible signs of stress on average 10 days later than the drought-stressed plants not grown in biochar. This finding adds to the already mounting evidence (Cao et al. 2014; Huang et al. 2020; Somerville et al. 2020; Yoo et al. 2020) that suggests the use of biochar is an effective technique to increase the resilience of plants to drought stress in urban forest management. Its use would have the immediate benefit of providing additional time to urban land managers to

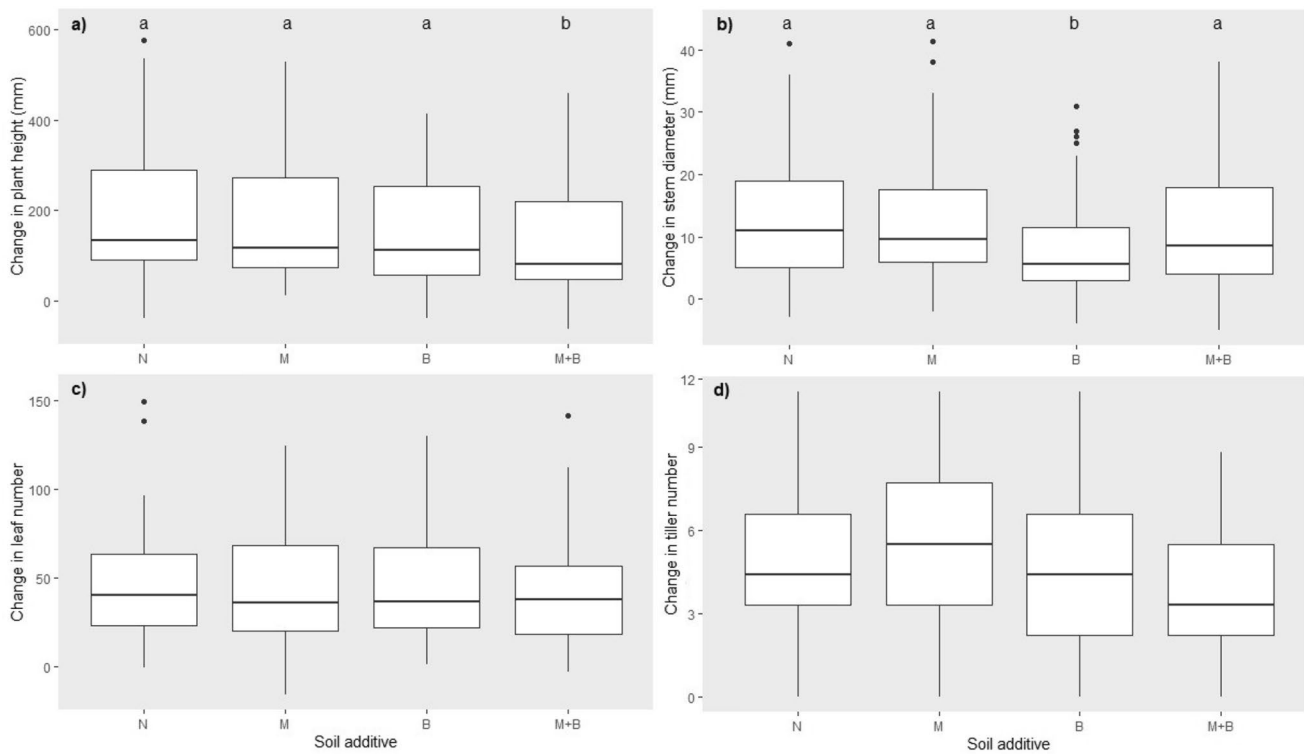


Fig. 4 The growth traits of the trees and graminoids for each soil additive treatment. Boxplots display the middle 50% of the data (interquartile range, IQR). Within the box, the horizontal bar represents the mean value. Vertical bars represent one standard error, while outliers are

indicated by dots. Letters indicate significant differences at $p < 0.05$. Soil additive treatment abbreviations: N = no additive, M = microbial additive only, B = biochar only, M + B = microbial additive and biochar

organise emergency irrigation for drought-stressed plants during drought periods. In the longer term, the negligible cost of biochar means that the benefit-cost ratio of using it is likely to be significantly greater than the plant maintenance/replacement costs that may otherwise be required.

It should be noted that the effectiveness of biochar in retaining soil water is strongly related to soil type and texture, with coarse-grained soils experiencing greater water retention than fine-grained soils (Jeffery et al. 2011; Omondi et al. 2016; Blanco-Canqui 2017; Razzaghi et al. 2020). Therefore, the degree to which biochar alleviates drought stress in field conditions may be dependent on these soil characteristics. For example, it has been reported that in fine-grained clayey urban soils, biochar reduced VSWC while in more coarse-grained sandy soils it has the opposite effect (Somerville et al. 2019). In our study, we used a coarse-grained potting mix (e.g. contained wood chip and vermiculite), so it is unsurprising that biochar significantly improved soil water retention.

The soil microbial additive we used did not significantly alleviate the negative impact drought stress had on the growth of our study species. However, when it was applied in combination with biochar, the plants had significantly greater mycorrhizal colonisation than the other soil additive

treatments irrespective of the watering regime. This suggests that the soil microbial additive enhanced the soil microbiome that was already present in the potting mix but the soil microbiome required the favourable environment created by the biochar (e.g. greater soil water availability, increasing carbon and nutrient supply, detoxification of allelochemicals and more refugia) to proliferate (Warnock et al. 2007; Palansooriya et al. 2019). Given that the diversity of the soil microbiome was limited to the microbes present in the soil microbial additive, it can be suggested that the addition of biochar enhanced microbial biomass rather than diversity. Although biochar typically increases soil microbial biomass (Pokharel et al. 2020), the biochar properties have been shown to have a significant influence (Li et al. 2020). Specifically, biochar produced at low temperatures (< 300 °C) from nutrient-rich feedstocks (e.g. manure, straw) typically result in the greatest increase in soil microbial biomass (Li et al. 2020). In comparison, the biochar used in this study was produced at 450 °C from wood and leaves. Although not ideal, it has been shown that biochar produced with these parameters significantly stimulates soil microbial biomass (Li et al. 2020). Therefore, the biochar production parameters may explain the increase in mycorrhizal colonisation

Fig. 5 The mycorrhizal colonisation for each (a) watering regime \times growth form combination and (b) soil additive treatment. Boxplots display the middle 50% of the data (interquartile range, IQR). Within the box, the horizontal bar represents the mean value. Vertical bars represent one standard error, while outliers are indicated by dots. Letters indicate significant differences at $p < 0.05$. Soil additive abbreviations: N=no additive, M=soil microbial additive only, B=biochar only, M+B=microbial additive and biochar

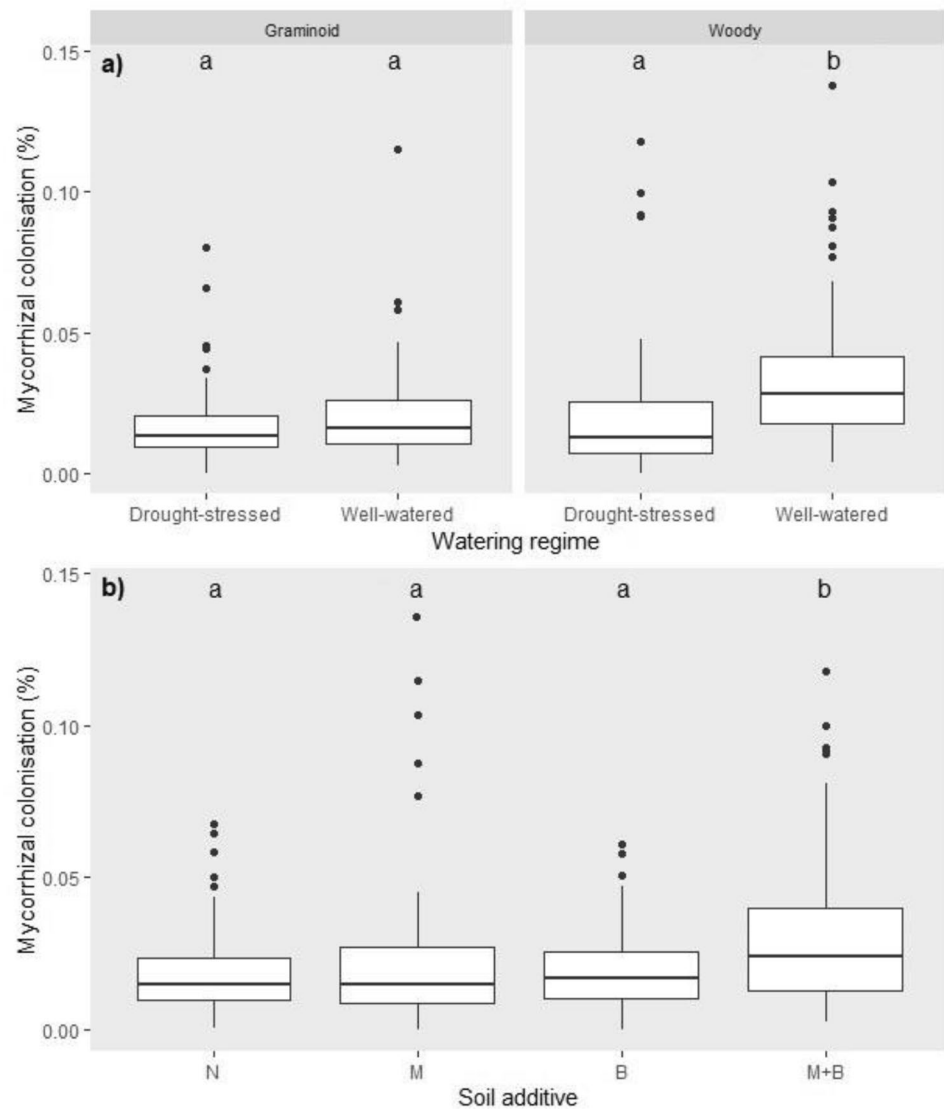
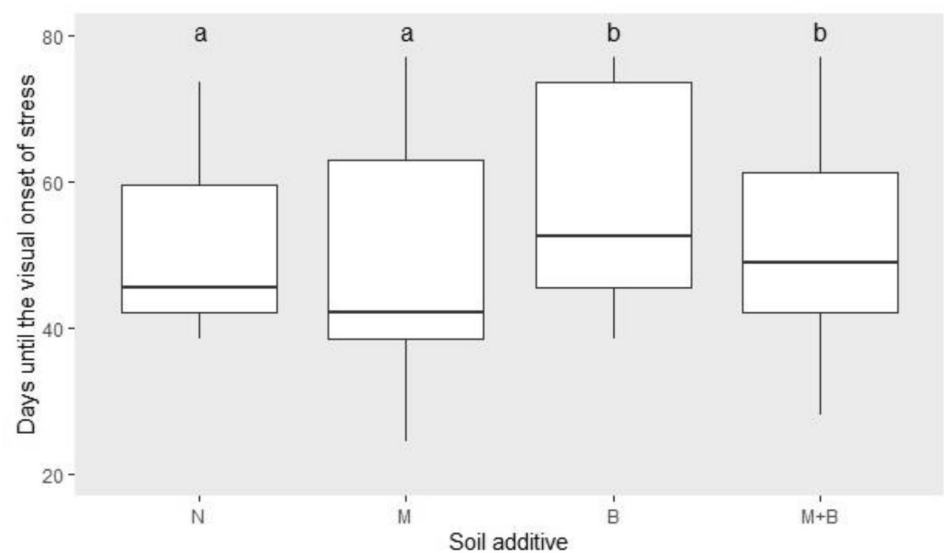


Fig. 6 Days until the visual onset of stress in the drought-stressed plants for each soil additive treatment. Boxplots display the middle 50% of the data (interquartile range, IQR). Within the box, the horizontal bar represents the mean value. Vertical bars represent one standard error, while outliers are indicated by dots. Letters indicate significant differences at $p < 0.05$. Soil additive treatment abbreviations: N=no additive, M=microbial additive only, B=biochar only, M+B=microbial additive and biochar



we observed when the soil microbial additive was applied in conjunction with biochar.

Although the increase in mycorrhizal colonisation when both soil additives were used in conjunction did not enhance the growth of our study species in the 12-week treatment period, it may facilitate growth in the long-term once mycorrhizal networks have become more established (Bonfante and Genre 2010). This is consistent with a recent review that suggests the positive benefits of biochar on the soil microbiome and the subsequent flow on effects to plant performance typically occur 1–6 months after application (Joseph et al. 2021). Further, the increase in mycorrhizal colonisation may have benefited the plants in other ways, as it has been shown that a healthy soil microbiome lessens the impacts of a range of abiotic (e.g. salinity, nutrient deficiency) and biotic stressors (e.g. pathogen attack) on plants (Rho et al. 2018; Wei et al. 2019; Poorter et al. 2020).

It needs to be acknowledged that several caveats should be considered when applying the findings of this study to the field. Firstly, we used an organic potting mix as our growth substrate instead of urban soil for reasons outlined in the Methods. Although mechanistically our findings should be applicable across different soil types, the effect of the soil additives on plant performance will likely vary depending on the soil (abiotic properties). Therefore, it is critical that a range of different urban soils are tested for us to gauge the consistency of the response. Secondly, our experiment was glasshouse-based with plants being grown in pots, which may not be representative of field conditions. However, the benefit of a glasshouse-based study is that you can eliminate confounding environmental factors that would exist in the field and focus on a targeted treatment, which in our case was soil water availability. Further, plants grown in urban areas are often faced with space constraints, albeit on a larger-scale, which means the response of pot-grown plants may be translatable to the field. Despite this, it is still important that field-based studies on the effect of soil additives on plant performance are carried out to validate the findings of glasshouse-based studies. Thirdly, our study as well as the literature examining the effects of soil additives on plant-water relations has focused on their potential beneficial outcomes, primarily when water is limited (Ali et al. 2017). However, it needs to be acknowledged that in certain contexts where water is not limiting, such as during extreme rainfall events, using soil additives may be detrimental to plant performance. For example, the use of soil additives that increase soil water retention may exacerbate hypoxia (oxygen deficiency) experienced by plants during intense or prolonged rainfall events, which can dramatically reduce their performance (Fukao and Bailey-Serres 2004). Further, plants growing in environments with high water availability, which the use of soil additives may facilitate,

tend to be more susceptible to pest outbreaks (Aung et al. 2018). Therefore, understanding the effects of soil additives on plant performance in urban forests with high water availability represents an important knowledge gap that needs to be addressed, as it may have implications for whether soil additives are appropriate to use in all or only certain urban areas.

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1007/s11252-023-01382-4>.

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Author contributions All authors contributed to the study's conception and design. Material preparation and data collection were performed by Manea A, Tabassum S, Lambert M and Cinantya A. Data analyses were performed by Manea A. The first draft of the manuscript was written by Manea A and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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Declarations

Ethics approval and consent to participate Not applicable.

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